NEW YORK UNIVERSITY–BELLEVUE MEDICAL CENTER
NEW YORK UNIVERSITY COLLEGE OF MEDICINE
550 FIRST AVENUE, NEW YORK 16, N.Y.

DEPARTMENT OF BIOCHEMISTRY

OREGON 9-3200

Dear Prof Lederberg

Thank you for your letter of sept 24. Some days ago I had a discussion with Dr. Ogiuski about streptomycin-resistant mutants. I do not agree that <u>all</u> streptomycin-R mutants have a respiratory defect, because I had many mutants with normal respiratory enzymes. But it is possible to get from E. coli porphyrin-negative mutants in the presence of streptomycine.

At the begining we have isolated from E. coli ML porphyrin-less mutant by transfer of the normal bacteria into liquid medium with increasing amounts of streptomycin sulfate. In this way, we had much difficulty to obtain it again. So we looked for a method to obtain regularly porphyrin-less mutants. As this mutant was sensitive to hemin, we have combined the action of hemin and streptomycin on the normal bacteria.

Techniques

<u>medium</u> : 20 gr peptone "Ucaf"
2 gr glucose
5 gr Na Cl
1000 ml H_2O, pH 7,4

<u>hemin solution</u> : 5 mg of hemin → 3 ml Na_2HPO_4 buffer $\frac{M}{2}$. Heating at about 70-80°C. After dissolution, complete the volume at 25 ml (H_2O). Sterilisation 10 min. at 105°C. Use always fresh solution (1-3 days) at 5°C.

<u>Streptomycin</u> sulfate has been used.

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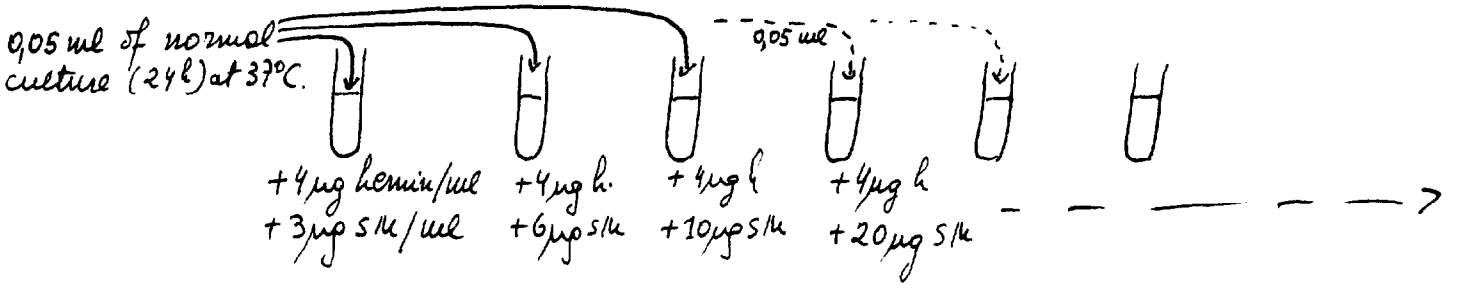
Essai tubes containing 10 ml of peptone water



After 24 h, the inoculum is taken in the tube with growth. So after some transfers into the next tubes we obtained a culture the optical density of which is more less then in the normal strain. Transferred on solid medium, the mutant gives very small colonies.

The same mode (with hemin) of isolation but with solid medium (Petri dishes) was unsuccessful.

We have obtained the porphyrin-negative mutants from E. coli ML ; B ; K-12 (C_{600}) ; but we could not get them from K-12 (112)*.

We do not know the mechanism of the appearance of such mutants. This problem has not been studied.

my best regards
M. Beljanski

* K-12 strains from Dr. Wollman and Jacob